DIAGNOSTICS

The use of serological titres of IgA and IgG in (early) discrimination between rectal infection with non-lymphogranuloma venereum and lymphogranuloma venereum serovars of *Chlamydia trachomatis*

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Objectives: To investigate whether serological titres of species-specific IgA and IgG antibodies in patients with rectal chlamydial infection could discriminate between infection with serovar L2 lymphogranuloma venereum (LGV) and infection with non-LGV serovars.

Methods: A total of 39 male patients with chlamydial infection of the rectum were tested for titres of IgA and IgG antibodies within 14 days after detection of the infection and 6 and 12 months after adequate treatment. Data were collected regarding demographics, sexual orientation, HIV serostatus, history of chlamydial infection, concomitant sexually transmitted infection (STI) or HIV infection, hepatitis C virus antibodies and new STIs during follow-up.

Results: Between May 2003 and November 2005, 24 men with confirmed L2 proctitis and 15 men with non-LGV rectal chlamydial infection were recruited. In multivariable analyses, both high titre of IgA within 14 days after detection of the infection and older age of the individual were found significantly associated with L2 proctitis ($p<0.001$ and $p=0.001$, respectively). A total sum score of seven times IgA titre and individual's age $\geqslant$50 years resulted in an overall sensitivity of 92% and specificity of 100%. This total sum score was highly accurate for detection of LGV proctitis, with an area under the curve in a receiver operating characteristic curve of 0.989.

Conclusions: An increased IgA antibody response and the age of the infected individual are of possible diagnostic value for (early) detection of LGV proctitis.

Chlamydia trachomatis infection is one of the most common sexually transmitted infections (STIs) in The Netherlands, with an estimated number of 60 000 cases annually.[1] *C trachomatis* comprises 15 classical serovars, serovars A–L and additional variants.[2] Lymphogranuloma venereum (LGV) is an STI caused by *C trachomatis* serovars L1, L2 and L3. LGV infection can be either symptomatic or asymptomatic.[3]

After the outbreak of LGV proctitis due to serovar L2 among Dutch men who have sex with men (MSM) in February 2003, a study in Rotterdam, The Netherlands, showed that these men present significantly more often with rectal symptoms and signs such as perianal erythema, discharge and loss of blood.[4] This is probably due to the severe, more invasive and more often chronic inflammation of the rectal mucosa and frequent involvement of pararectal lymph nodes in cases of infection with the more invasive L2 serovar compared with infection with other serovars.

In patients with a microbiologically confirmed rectal chlamydial infection, self-reported rectal symptoms and signs can be inadequate as a predictor of LGV. Genotyping is an accurate but expensive, time-consuming and not readily available procedure. In The Netherlands, discrimination between LGV and non-LGV is currently performed routinely at only two laboratories.

Alternatively, both clinical picture (symptoms and signs) and high titres of serum IgA and IgG antibodies can facilitate, in an early stage of the diagnostic process, discrimination between rectal *C trachomatis* infections caused by LGV and non-LGV serovars.[3 5]

In our view, treating all rectal *C trachomatis* infections with doxycycline 100 mg orally twice daily for 3 weeks is not an option. According to current recommendations, non-LGV serovars can be treated equally efficaciously with single-dose azithromycin (1 g orally), with similar tolerability and with higher compliance.[6] Furthermore, the Centers for Disease Control and Prevention currently state that azithromycin should always be available for patients for whom compliance with multi-day dosing is in question.[7]

Different studies have suggested that the humoral immune compartment of the human genital tract shows features that are functionally different from those of other compartments of the mucosal immune system, such as the gastrointestinal tract.[8 9] In contrast to the predominance of IgG-producing cells in the human genital tract, the intestinal tract, including the proctum, contains a much higher proportion of IgA-producing cells.[10] Because of the presence of special inductive lymphoepithelial structures, the rectal immune system induces both local and generalised immune responses, manifested in the parallel appearance of IgA antibodies at the site of exposure and in anatomically remote mucosal tissue.[11]

The aim of this study was to investigate whether serological titres of species-specific IgA and IgG antibodies in patients with rectal chlamydial infection could discriminate between infection with serovar L2 LGV and infection with non-LGV serovars.

Abbreviations: IL, interleukin; LGV, lymphogranuloma venereum; MSM, men who have sex with men; OD, optical density; STI, sexually transmitted infection

MATERIALS AND METHODS

Study population and study design

This study was conducted at the STI clinic of the Department of Dermatology and Venereology, Erasmus MC Rotterdam, The Netherlands. Since the outbreak of proctitis resulting from LGV in MSM in February 2003, all rectal chlamydial infections detected at the STI clinic were genotyped.[6 12] All patients attended the STI clinic on their own initiative because of sexual risk behaviour or symptoms possibly related to STI. Patients underwent a standardised venereological examination as described previously.[13] Because of a recently detected cluster of acute hepatitis C virus infection among MSM at the STI clinic, testing for hepatitis C is routinely performed in all patients with LGV proctitis and in those with rectal symptoms suspicious for LGV proctitis.[14]

Patients who provided written informed consent for this study were tested for the *C trachomatis* antibodies IgA and IgG within a maximum of 14 days after detection of the rectal chlamydial infection. Blood testing for C reactive protein took place as well. Treatment of the infection was started after taking the blood samples. All non-LGV *C trachomatis* infections were treated with azithromycin 1 g orally in a single dose and LGV infections with doxycycline 100 mg orally twice daily for 3 weeks. Post-treatment swabs were taken only in patients with LGV. All infections with LGV completely cleared after treatment with doxycycline.

In HIV-positive individuals, blood samples were taken for a CD4 lymphocyte count, or recent (not older than 6 months) blood counts were used.

After 6 and 12 months of enrolment, *C trachomatis* IgA and IgG antibodies were tested again. Because of the small number of included patients who did return after 6 months and 12 months for their antibody blood test, analyses were only performed for the initial IgA and IgG titres tested at enrolment. Although we sent letters and e-mails to all those who did not revisit the STI clinic, more than half of the patients in both groups did not return for their scheduled appointment.

Data collection

Demographic and sexual behavioural data were collected. These included sex, age, ethnic background, sexual orientation and earlier diagnoses of STI, including HIV infection. Also, data concerning newly detected STI or HIV infections during the follow-up period were collected.

Genotyping

To detect *C trachomatis* DNA in clinical specimens, the automated *C trachomatis* Cobas Amplicor PCR system (Roche Diagnostic, Almere, The Netherlands) was used throughout the study, in accordance with the instructions of the manufacturer.

Rectal swabs were collected in 2SP medium and subsequently used for PCR testing. Genotyping of the gene encoding the major outer membrane protein was performed by nested PCR and restricted fragment length polymorphism analysis.[2 12 15] Results were reported as ''LGV infection'' (serovars L1, L2 or L3) or ''non-LGV-infection'' (one of the 15 classical *C trachomatis* D–K serovars).

Antibodies to *C trachomatis*

Sera of all patients were tested for IgA and IgG antibodies to *C trachomatis* using a peptide enzyme immunoassay (SeroCT; Savyon Diagnostics, Ashdod, Israel). Results were reported as the ratio of the optical density (OD) of the serum specimen to the OD of the cut-off control specimen. Specimens with reactivity beyond the maximum of the spectrophotometer were reported as larger than the highest measurable value. Thus, specimens could be classified into one of three groups: (1) negative, (2) measurable ratio and (3) greater than the maximal measurable ratio. This maximum varied between runs, as the OD value of the cut-off control specimen varied slightly.

Statistical methods

Univariable and multivariable logistic regression analyses were used to investigate the association between individual's age, titres of IgA and IgG antibodies, and LGV infection versus non-LGV infection. A test was considered significant if the p value was <0.05.

To calculate the diagnostic accuracy of the titres of IgA and IgG, further analyses were performed. When a specimen with reactivity beyond the maximum of the spectrophotometer was detected, the highest measurable value was used in our analyses. A weighted sum score was computed using the linear predictor calculated with the coefficients (log odds ratios) of logistic regression analyses as weights.

Receiver operating characteristic (ROC) curves were composed to find the cut-off point for optimally classifying persons with or without LGV. The ROC curve is a plot of the sensitivity, or true-positive rate, to the false-positive rate (1−specificity). The area under the curve (AUC) measures the ability of the score to correctly classify persons with or without disease. The closer a ROC curve is to the upper-left hand corner of the graph, the more accurate it is (AUC = 1). An ROC curve equal to the 45° line of identity corresponds to a test result that is positive or negative only by chance (AUC = 0.5). A test with an ROC curve with an AUC of >0.75 is considered to be accurate.

All statistical analyses mentioned in this study were performed using SPSS for Windows, V.12.0.

RESULTS

Between May 2003 and November 2005, 24 men with confirmed L2 proctitis and 15 men with rectal chlamydial infection, serovars D–K, were included in this study.

In all, 34 (87%) of the patients identified themselves as homosexual, and 5 (13%) described their sexual orientation as bisexual. The median age of the men was 38 years (interquartile range 29–42), and 29 (74%) men were of native Dutch descent. Men in the LGV group were significantly older than men in the non-LGV group (p = 0.008). Table 1 summarises the demographic data for both groups of patients investigated in this prospective study.

With respect to HIV serostatus, 27 (69%) men were known to be HIV-positive at enrolment. In the LGV group, 20 (83%) men were known to be HIV-positive, compared with 7 (47%) men in the non-LGV group (p = 0.057). Of these 27 HIV-positive men, 3 (13%) men in the LGV group had newly detected HIV-coinfections, compared with 5 (33%) men in the non-LGV group (p = 0.12). CD4 counts were slightly, but not significantly, higher in the LGV group (p = 0.22). Only two patients in the LGV group and one in the non-LGV group had CD4 counts $\leqslant 200$ cells/μl, representing severe immune suppression (p = 0.49).

A concomitant STI was detected in 8 (33%) men in the LGV group compared with 6 (40%) men in the non-LGV group (p = 0.67). Antibodies against hepatitis C were detected in four men in the LGV group. Antibodies against hepatitis C could not be found in non-LGV men ($p<0.001$). However, not all men in this group were tested.

During the first year of follow-up, 5 (23%) men in the LGV group and 1 (8%) man in the non-LGV group acquired a new STI or were HIV-seroconverted (p = 0.16). None of these new infections were chlamydial infections. Some of these newly detected infections (syphilis: three times) could have been a

Table 1 Demographics, sexual orientation, HIV status, concomitant sexually transmitted infection (STI) and titres of IgA and IgG antibodies in patients with rectal *Chlamydia trachomatis* infection attending the Erasmus MC STI clinic

	Non-LGV	LGV	p Value
Patients (n)	15	24	
Age (years), median (IQR)	34 (26–38)	39 (34–43)	0.008
Ethnicity (Dutch)	11/15 (73%)	18/24 (75%)	0.91
Sexual orientation			0.30
Male homosexual	12/15 (80%)	22/24 (92%)	
Male bisexual	3/15 (20%)	2/24 (8%)	
HIV serostatus			0.057
HIV positive	7/15 (47%)	20/24 (83%)	
HIV negative	6/15 (40%)	4/24 (17%)	
Unknown	2/15 (13%)	—	
CD4 count (in cells/μl), median (IQR)	370 (370–520)	525 (420–655)	0.22
History of chlamydial infection	3/15 (20%)	6/23 (26%)	0.85
Concomitant STI	6/15 (40%)	8/24 (33%)	0.67
Antibodies against HCV	0/5 (0%)	4/24 (17%)	<0.001
Newly detected HIV infection	5/15 (33%)	3/24 (13%)	0.12
New STI within first year	1/12 (8%)	5/22 (23%)	0.16
CRP at the time of detection, median (IQR)	1 (1–3)	2 (1–14)	0.073
IgA at the time of detection, median (IQR)	0.6 (0.5–1.0)	3.5 (2.3–5.0)	<0.001
IgG at the time of detection, median (IQR)	2.3 (0.7–3.7)	5.3 (4.0–6.9)	<0.001
IgA specimen classification ''negative''	11/15 (73%)	2/24 (8%)	<0.001
Measurable ratio	4/15 (27%)	15/24 (63%)	0.027
Greater than measurable ratio	0/15 (0%)	7/24 (29%)	0.005
IgG specimen classification ''negative''	5/15 (33%)	0/24 (0%)	0.001
Measurable ratio	9/15 (60%)	5/24 (21%)	0.013
Greater than measurable ratio	1/15 (7%)	19/24 (79%)	<0.001
	n = 10	n = 17	
IgA after 6 months, median (IQR)	0.4 (0.3–1.1)	3.1 (1.2–5.3)	<0.001
IgG after 6 months, median (IQR)	1.5 (1.1–3.2)	5.4 (4.2–6.6)	0.001
	n = 6	n = 9	
IgA after 1 year (median, IQR)	0.7 (0.5–0.9)	4.3 (0.9–6.1)	0.003
IgG after 1 year, median (IQR)	2.2 (1.2–3.5)	5.4 (3.7–6.5)	0.012

CRP, C reactive protein; HCV, hepatitis C virus; IQR, interquartile range; STI, sexually transmitted infection.

prevalent infection not detected earlier because of the window period.

An earlier diagnosis of *C trachomatis* was reported by 6 (26%) men in the LGV group and by 3 (20%) men in the non-LGV group ($p = 0.85$). The exact site (urethral or rectal) of these earlier infections was not known.

IgA and IgG antibodies

Table 1 summarises titres of IgA and IgG antibodies in the group of patients investigated in this study.

Men with L2 proctitis had significantly higher titres of species-specific IgA and IgG after detection of their infection ($p<0.001$). Titres of species-specific IgA and IgG were still significantly increased in men with a former L2 proctitis ($p<0.01$) 6 and 12 months after treatment.

Diagnostic accuracy

In multivariable logistic regression analyses, individual's age ($p<0.001$) and titres of IgA antibodies ($p = 0.001$) were found to be significantly associated with type of infection (LGV infection vs non-LGV infection). Titres of IgG antibodies ($p = 0.23$) did not contribute to this association.

ROC curves showed that a linear combination of IgA titre and individual's age (weighted sum score) had more accuracy for detection of LGV infection than either IgA titre or individual's age alone. This weighted sum score (4.4×IgA titre plus 0.6×individual's age) had a relevant accuracy, with an AUC of 0.989 (SE = 0.012). When a more convenient total sum score of both IgA titres and individual's age (7×IgA titre plus individual's age) was used, accuracy was identical to the accuracy for detection of LGV using the weighted sum score (AUC = 0.989; SE = 0.012). Using IgA titre or individual's age alone, accuracy was lower (AUC = 0.950; SE = 0.031 and AUC = 0.735; SE = 0.084, respectively). No significant interaction could be found between the titres of IgA and IgG ($p = 0.92$) or between IgA titre and individual's age ($p = 0.66$).

A total sum score of 7×IgA titre plus individual's age ⩾50 years results in an overall sensitivity of 92% and a specificity of 100%. Figure 1 is a scattergram of IgA titre and individual's age, including the cut-off line (total sum score ⩾50). Table 2 shows the fraction of LGV cases detected (sensitivity) and fraction of LGV-negative cases not detected (specificity) in relation to the various cut-off points of the total sum score.

DISCUSSION

The aim of this study was to investigate whether serological titres of species-specific IgA and IgG antibodies in patients with rectal chlamydial infection could discriminate between infection with serovar L2 LGV and infection with non-LGV serovars.

At the time of detection and 6 and 12 months after adequate treatment of the *C trachomatis* infection, men with rectal LGV had significantly higher titres of species-specific IgA and IgG antibodies. These significantly increased titres, which can

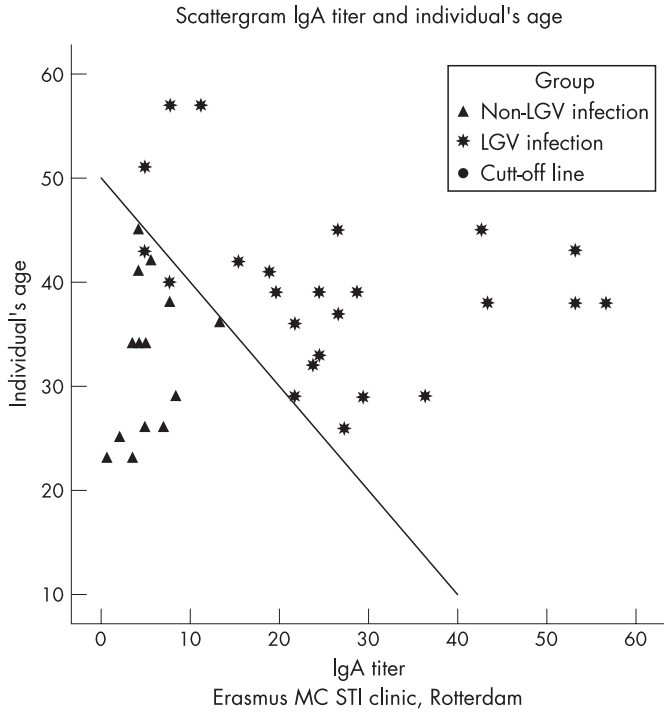

Figure 1 Scattergram of IgA titre and individual's age with cut-off line (total sum score ⩾50). LGV, lymphogranuloma venereum.

slowly diminish over time, probably represent the severe, more invasive and more often chronic inflammation of the proctum caused by LGV serovars, compared with other serovars.

ROC curves showed that the use of a total sum score of seven times IgA titre plus individual's age had a high diagnostic accuracy for detection of LGV proctitis. This total sum score (7×IgA titre plus individual's age) is an easy-to-use instrument in the diagnosis of LGV proctitis. A total sum score ⩾50.0 results in a specificity of 100% and a sensitivity of 92%.

It is unlikely that differences in the number of concomitant HIV infections or anti-hepatitis C virus antibody positivity in both groups can explain the increased antibody response in L2 proctitis as seen in this study. A lower antibody response could perhaps be expected in HIV-positive, severely T–cell-depleted patients. In this study, only 3/27 (11%) patients had CD4 counts ⩽200 cells/μl, representing relevant immune suppression.

Microbiological and immunological observations from in vitro studies suggest some explanations for the differences between serological titres of species-specific IgA and IgG antibodies in patients with rectal chlamydial infection caused by serovar L2 LGV and infection with non-LGV serovars. From a microbiological point of view, both LGV and non-LGV serovars enter epithelial host cells at the apical surface as infectious elementary bodies, and, once inside the cell, remain there within membrane-bound vesicles. In case of multiple vesicles, they usually fuse to form one large vesicle. These elementary bodies transform into metabolically active, non-infectious forms called reticulate bodies. Later in the development cycle, the reticulate bodies condensate into infectious elementary bodies and are released from the host cell to infect neighbouring cells. The host cell cytoskeleton plays a unique role in the uptake and development of the different serovars. LGV serovars might be able to leave the host cell at the basolateral surface to infect cells under the epithelial layer. Inclusions from non-LGV serovars remain after maturation in the apical domain, where they eventually escape from the host cells to infect the apical surfaces of neighbouring cells.[16]

Table 2 Test characteristics of different total sum scores

Cut-off total sum score*	Sensitivity† (95% CI) n = 24	Specificity‡ (95% CI) n = 15
54.6	83.3 (57.9 to 92.9)	100 (78.2 to 100)
52.0	87.5 (67.6 to 97.3)	100 (78.2 to 100)
50.0	91.7 (73.0 to 99.0)	100 (78.2 to 100)
49.3	91.7 (73.0 to 99.0)	93.3 (68.1 to 99.8)
48.6	91.7 (78.9 to 99.9)	86.7 (59.5 to 98.3)

*Cut-off total sum score: 7×IgA titre plus individual's age.
†Sensitivity: the percentage of LGV infections detected under a given selection.
‡Specificity: the percentage of LGV-negative participants not detected under a given selection.

From an immunological point of view, the innate immune response to host cells infected with serovar L2 LGV is different from the innate immune response after infection with non-LGV serovars. This response is thought to be initiated by the release of proinflammatory cytokines from the infected cells. L2 serovar-infected cells seem to produce larger amounts of interleukin (IL)11, which inhibit the release of proinflammatory mediators such as tumour necrosis factorα, IL1β, IL6, IL12 and nitric oxide from activated macrophages, as well as the production of IL12 from dendritic cells. The immunosuppressive effects of IL11 might allow L2 to escape host innate defenses for better dissemination, while the extended infection eventually causes a higher humoral immune response, because of the severe, more invasive and more often chronic inflammation of the proctum caused by L2 compared with other serovars.[17]

The small sample size of this study does not influence sensitivity or specificity of our total sum score (7×IgA titre plus individual's age) instrument in the diagnosis of LGV proctitis. In a sample size with lower a priori chance for LGV proctitis, the positive predictive value of the total sum score will be as high as 100%, because of a specificity of 100%. Nevertheless, we advise to test the sera of individuals in high-risk populations only.

It is important to note that, in our study, IgA and IgG assays from one commercial company were used. Other manufacturers produce similar enzyme immunoassays, but with different peptides. These assays might not produce the same results, although similar results might well be expected.

We conclude that, in this study, increased IgA antibody response and the age of the infected individual seemed to be of possible diagnostic value for (early) detection of LGV proctitis.

Key messages

- Rectal chlamydial infections with serovar L2 are associated with significantly higher titres of IgA and IgG antibodies, probably because of more invasive and more frequent chronic inflammation of the proctum.
- In this study, conducted at the Rotterdam sexually transmitted infection clinic, data generated from examining a limited patient population showed that an elevated IgA antibody response and the age of the infected individual were of possible diagnostic value for (early) detection of lymphogranuloma venereum proctitis.
- A total sum score of seven times IgA titre plus the individual's age ⩾50 resulted in an overall sensitivity of 92% and a specificity of 100%.

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